Remarks/Arguments

Claims 1-25 and 27-46 are currently pending in the instant application. In view of the finality of the restriction requirement, claims 1-25 are under examination and claims 27-46 have been withdrawn from consideration. Claim 1 is amended with the present Response. The amendment is supported throughout the specification and is intended to establish consistency with element (a) of claim 1. No new matter is added by way of this amendment and its entry is respectfully requested.

Claims Rejections – 35 USC § 103

Claims 1-25 stand rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Genentech (Basey et al.; WO 99/57134) in view of Grandics et al. (US 5,571,720) and newly added reference Winge (US 2001/0034053 A1).

Basey et al. is cited for teaching protein purification by ion exchange chromatography. The Examiner acknowledges that Basey et al. do not teach an ion exchange chromatography system where a salt gradient, not to mention non-linear salt gradient, is employed, and cites Grandics et al. as allegedly filling this void. In particular, Grandics et al. is cited as allegedly teaching the use of an integrated cell culture protein purification system using ion exchange chromatography with a salt gradient. In responding to Applicants' arguments submitted in response to an earlier similar rejection, the Examiner asserts that Grandics et al. actually teach a non-linear salt gradient option in columns 7 and 8. (Office Action, page 7, 1st paragraph) Winge is relied on for allegedly describing at para's 42-43, 63, and 81, his version of "protein purification via cation exchange chromatography employing different salt gradients resulting in a non-linear salt gradient" (Office Action, pages 4 and 5), and thus providing express teaching regarding one version of "protein purification via cation exchange chromatography employing different salt gradients resulting in a non-linear salt gradient." (Office Action, page 6, 1st paragraph)

The rejection is respectfully traversed.

The finding of obviousness appears to be based on the assumption that the only feature distinguishing the invention claimed in the present application is the use of a non-linear salt

gradient, which, according to the rejection, was known in the art at the time the present invention was made. However, this is not the case.

The invention disclosed and claimed in the present application is a method for purifying a polypeptide from a composition comprising the polypeptide and contaminants, which method comprises a number of sequential steps, namely (a) loading the composition onto an ion exchange resin with an equilibration buffer having a first salt concentration; (b) washing the ion exchange resin with a wash buffer until a predetermined protein concentration is measured in the flowthrough, wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration; (c) passing a fixed volume of wash buffer at the final, third salt concentration over the ion exchange resin; and (d) eluting the polypeptide from the ion exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer (see claim 1). Thus, the claims recite a sequence of steps, the relationship of salt concentrations employed in the various steps to each other, and the timing of performing washing step (b).

As described in the specification, the inventors found that the instantly claimed wash steps, performed in the order and using the parameters recited in the claims, were effective for polypeptide purification, particularly for resolving a desired polypeptide molecule from a contaminant differing only slightly in ionic charge.

As is recognized in ion exchange chromatography, the more similar the charge on the two molecules to be separated (polypeptide vs. contaminant), the more difficult it is to achieve a separation of the two molecules. The experimental examples in the patent application illustrate the use of the claimed methods to substantially reduce the amount of deamidated and other acidic variants in a purification of anti-HER2 antibody, which is a technically challenging separation owing to the very similar properties of the variants compared with the desired antibody polypeptide. In Example 1, ion exchange chromatography was performed using a linear salt gradient (shown in Figure 2), followed by a wash with 1 column volume (CV) of the wash buffer at the final salt concentration. In Example 2, ion exchange chromatography was performed using

a multi-slope gradient (shown in Figure 3), followed by a wash with fixed volumes of 0.4, 0.6 or 0.8CV.

In discussing the results from Example 2 it is stated on page 33 lines 5-8 that:

> The results presented in Tables 4 and 5 show that it is possible to achieve consistent yield and acidic variant removal over a wide load range by using a multislope gradient wash. In addition, the results demonstrate that the trade off between yield and purity can be fine tuned as desired by adjusting the end-wash delay volume after an OD of 0.6 is achieved.

The "end-wash delay volume" referred to here is the wash using a volume of buffer at the final salt concentration or conductivity prior to elution, which links the wash step to an analytical measurements of the flowthrough. The effect of this wash is discussed in the following paragraph (page 33 lines 9-13), describing its ability to increase purity of the eluted protein.

The methods claimed in the present application are not disclosed or suggested by the cited combination of references, and represent a significant contribution to the art in view of the demonstrated unexpected effects achieved.

The combination of references cited does not teach or suggest all limitations of the pending claims.

As acknowledged by the Examiner, Basey et al. teach protein purification by ion exchange chromatography, but do not teach an ion exchange chromatography system where a salt gradient is employed.

Grandics et al. teach the use of an integrated cell culture protein purification system using an ion exchange chromatography using a single linear salt gradient. However, Grandics et al. do not teach applying a salt gradient of any kind, linear or non-linear, during the washing phase of the purification method. It is only when the "product is recovered", or during the elution phase, that Grandics contemplates applying a low salt buffer or a reverse salt gradient. The Examiner's assessment ignores a significant difference between the washing and eluting steps in chromatography. The claimed methods rely on washing with an increasing salt concentration to remove contaminants while the protein of interest remains bound to the column, until a

predetermined protein concentration is measured in the flowthrough, at which time the protein of interest is eluted off of the column at a final salt concentration that is greater than the wash buffer. By contrast, the method disclosed in Grandics is washing at a single salt concentration that is higher than the elution buffer, followed by elution at a salt concentration lower than the wash buffer or over a decreasing salt gradient. Thus, contrary to the Examiner's assertion, Grandics et al. do not provide the information missing from Basey et al. and therefore the combination of Grandics et al. with Basey et al. does not teach the invention claimed in the instant claims. The addition of Winge et al., representing general background art, to the combination does not change this conclusion.

The passages cited by the Examiner from Winge et al. only generally refer to the use of salt gradients with ion exchange chromatography and teach that "[e]lution of proteins can be carried out by stepwise higher salt concentrations or by linear or non-linear salt concentrations." Winge et al. do not disclose or suggest the specific application of salt gradients in the washing step as claimed in the instant application, or the sequence of steps and process parameters recited in the pending claims. As discussed above for Grandics et al., Winge et al. do not teach applying a salt gradient of any kind, linear or non-linear, during the washing phase of the purification method. In all specific examples provided by Winge (Example 1 and 2, pages 5-7), a single salt concentration was used in the wash step and its duration was based on a predetermined volume of buffer being passed through the column. By contrast, step (b) in claim 1 requires washing at increasing salt concentration until a predetermined protein concentration is measured in the flowthrough.

The claimed method of washing "until a predetermined protein concentration is measured in the flowthrough" is a very different approach compared with the teaching in the cited prior art, which employ washing with a defined volume of buffer. In the cited methods, the skilled person applies a set volume of buffer, and there is no teaching linking the wash step to any analytical measurements of the flowthrough. In contrast, in the claimed method the wash buffer is added until the predetermined protein concentration is measured in the flowthrough, so the composition and application of wash buffer in the claimed method thus depends on the output. In addition, the increase in salt concentration of the wash buffer from a second to a third salt concentration is

not disclosed in any of the cited prior art references, when taken alone or in any combination. Instead, the prior art documents use a single salt concentration during the washing step.

Applicants note that the Examiner appears to use very general terms in applying the prior art, such as disclosure of "non-linear" salt gradients. Despite the fact that the cited art does not disclose such gradients in the washing step, the instant method more specifically teaches what might be referred to as a multi-slope gradient. Further distinguishing the instant method from cited combination of references, Claims 12 and 13 specifically disclose that the percentage of elution buffer in the wash buffer increases at two or more different rates during the course of washing and/or at a first rate for a first segment of the washing, at a second rate for a second segment of the washing and at a third rate for a third segment of the washing, respectively.

Accordingly, the cited combination of references does not teach the invention claimed in rejected claims 1-25.

In addition, there is no motivation or suggestion to modify the cited references to reach the Applicants invention

As discussed above, the cited prior art does not teach or suggest the methods of claim 1-25, which involve washing with an increasing salt concentration and then washing with a volume of wash buffer at the final salt concentration "until a predetermined protein concentration is measured in the flowthrough." Nor does the use of a salt gradient elution in the cited art teach or suggest the multi-slope wash procedure of claims 12 and 13. In summary, the wash steps used in the methods of the present invention are very different from the simple step gradient wash described in Basey, Grandics or Winge and these references do not provide any suggestion to the skilled person to employ the wash steps as presently claimed.

The Examiner further asserts that "the use of the salt gradient for purifying any protein as in Basey et al. would have merely been a matter of optimization by a protein research scientist versed in the methods of protein purification, as guided by the results sought." (page 6 of the instant Office Action)

Applicants submit that the cited art does not offer suggestions or even recognize a need for modification of their respective methods. In the absence of any recognized failure or shortcomings of these cited methods, the Examiner has provided no motivation for a protein research scientist to pursue any modifications, let alone provide a reasoning for selecting the specifically claimed method of the instant application over the multitude of possible salt gradient combinations possible.

With regard to Grandics, Applicants submit that Grandics teaches the application of a low salt buffer or a reverse salt gradient. While this is common practice in purification methods using hydrophobic interaction columns, it is unsuitable for applications using ion exchange chromotagraphy. In suggesting that one skilled in the art would combine the teachings of Basey et al., using ion exchange chromotagraphy, and Grandics, using a method reversing the salt concentration from high to low during the washing and elution steps, the Examiner has erred in resolving the level of skill in the art. A person skilled in the art would not make this combination. Hence, there would be no motivation to apply the reverse salt gradient method of Grandics to achieve the instant method. Further, even if one were to consider the reverse salt gradient of Grandics, it would clearly result in an approach different from the instant method which comprises washing the ion exchange resin with a wash buffer, "wherein the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration, and eluting the polypeptide from the ion exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer." (Claim 1 of the instant application)

The advantage that the instant method provides over the prior art is that, by using a three slope gradient wash, a greater range of resin load densities are possible while maintaining product quality and not sacrificing yield. The separation also becomes less sensitive to small variations in buffer composition. Washing steps are often employed to remove a contaminant from the column prior to elution, however, the resolution of species that a gradient wash would technically provide would not appear valuable to one skilled in the art since those species will never be collected. Moreover, multi-slope gradients are very complicated to run in a

manufacturing environment and it would strike the unfamiliar process developer as pointless to incorporate such a complicated operation into a wash phase that will be discarded. As discussed above, development of the multi-slope gradient used in the instant method was the solution to the problem of achieving a wide resin load density range while maintaining yield and purity. The achievement of this improvement in the load density range is a significant improvement over the prior art. A complicated series of interactions occur within the ion exchange column during the wash step and acidic variants are essentially just teased off the resin charge groups. At low resin load densities there are a large number of unoccupied resin binding sites that the acidic variants can loosely re-associate with, which means that from one run to the next, as the amount of protein loaded on the column varies, it can take a longer amount of time or require greater conductivity to get the acidic variants off the column. The three slope gradient of the instant method is a novel and non-obvious solution to this problem. Together the multi-slope gradient wash followed by the step elution provides a method to consistently remove acidic variants with a robust, reproducible process that can be effectively automated and scaled up. The prior art taken alone or in combination would not lead a skilled artisan to apply the multi-slope gradient to the wash step as instantly claimed.

Finally, Applicants maintain that the Examiner's conclusion of obviousness is based upon improper hindsight reasoning. As discussed above, the references cited by the Examiner do not teach or suggest protein purification via ion exchange chromatography employing washing steps with an increasing salt concentration to remove contaminants while the protein of interest remains bound to the column, until a predetermined protein concentration is measured in the flowthrough, at which time the protein of interest is eluted off of the column at a final salt concentration that is greater than the wash buffer. In addition, there is no motivation or suggestion to modify Basey et al., Grandics et al. or Winge et al. to reach the present invention, and no reasonable expectation that a combination of the reference with knowledge in the art would succeed in producing a protein purification method via ion exchange chromatography that achieves the claimed method. Thus, the only way the Examiner might have arrived at combining the cited references is using the disclosure of the present application to pick and choose selected portions of Basey et al., Grandics et al. and Winge et al., anion exchange chromatography, salt gradients and non-linear gradients respectively, in an attempt to recreate the claimed invention.

Applicants respectfully submit that the Examiner's conclusion is based upon an improper hindsight reconstruction of the claimed invention, which, instead of looking at the prior art as a whole, picks and chooses teachings that appears to support a finding of obviousness, while completely disregarding others.

In summary, Applicants have shown that that the Examiner has not established a *prima facie* case of obviousness because: (1) the references cited by the Examiner do not teach or suggest protein purification via ion exchange chromatography employing different salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough. (2) there is no motivation or suggestion to modify the reference to reach the instant protein purification method because the references do not discloses protein purification via ion exchange chromatography employing different salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough. (3) there is no reasonable expectation of success that a combination of the references would succeed in producing a protein purification method utilizing ion exchange chromatography and non-linear salt gradients due to the lack of disclosure of washing steps employing different salt gradients during the washing phase, wherein the washing involves increasing salt concentration until a predetermined protein concentration is measured in the flowthrough.and (4) the Examiner has based obviousness on improper hindsight. Accordingly, Applicants request that the rejection of Claims 1-25 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Basey *et al.* in view of Grandics *et al.* and further in view of Winge *et al.* be withdrawn.

Conclusion

The present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 50-4634 **(Attorney Docket No.: 123851-181807 (GNE-0113.A))**. Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: September 29, 2009

By _____
Christopher De Vry (Reg. No. 61,425)

GOODWIN PROCTER LLP
135 Commonwealth Drive
Menlo Park, California 94025
Telephone: (650) 752-3100
Facsimile: (650) 853-1038

LIBC/3680522.1